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(71) Applicant: GLYKO, INC. [US/US]; 81 Digital Drive, Novato, CA 94949 (US).

(72) Inventor: KLOCK, John, C., Jr.; 345 Willow Road, Box 611, Nicasio, CA 94946 (US).

(74) Agents: HALLUIN, Albert, P. et al.; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).

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(54) Title: METHODS AND COMPOSITIONS FOR TREATING DISEASES WITH CARBOHYDRATE MODIFYING ENZYMES

(57) Abstract

The invention provides methods and compositions for the treatment and/or prevention or a variety of disease conditions. The methods involve the administration of an effective amount of a therapeutic catalyst to a patient. A therapeutic catalyst is an enzyme, or other catalyst, capable of catalyzing a chemical reaction employing a carbohydrate or a carbohydrate portion of a glycoconjugate as a substrate. Therapeutic catalysts are selected so as to catalyze reaction that results in the structural alteration of at least one member of a receptor binding pair of molecules, whereby the interaction between receptor binding pair members is disrupted. The invention also provides compositions containing therapeutic catalysts for use in the subject methods. Diseases that may be treated by therapeutic catalysts include infectious diseases, cancer, and autoimmune diseases.

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METHODS AND COMPOSITIONS FOR TREATING DISEASES WITH CARBOHYDRATE MODIFYING ENZYMES

Field of the Invention

The subject invention is in the field of medical therapeutics, in particular, the field of therapies aimed at altering the carbohydrate structures naturally in the body.

Background of the Invention

Carbohydrates play a number of extremely important roles in living organisms. In addition to their roles as metabolic fuels and energy storage materials, carbohydrates may serve as binding and recognition signals. Carbohydrates may exist as free molecules in the body or carbohydrates may be covalently attached to numerous other molecules such as proteins (glycoproteins) and lipids (glycolipids). Molecules such as glycoproteins and glycolipids are generally referred to as glycoconjugates.

The carbohydrate portions of glycoconjugates may comprise up to 5-10% of the dry weight of a living cell, tissue or organ in the body. Many of these carbohydrates are located on the outside contact surface of body cells, tissues, or organs where they communicate and exchange signals or information with other cells, tissues, or organs. The body has a complex collection of enzymes that are responsible for the maintenance and recycling of these carbohydrates. Some of these enzymes have carbohydrate-splitting, i.e., hydrolysis, actions; some enzymes have carbohydrate-buildup actions, and

some have the ability to chemically "shield", "coat", or cover the surface of a carbohydrate to mask or modify its biologic activity. This array of carbohydrate-modifying enzymes also has the ability to modify or correct the chemical structure of the body's carbohydrates so as to prevent disease or to counteract diseases once they are established.

The biological importance of the carbohydrate portion of glycoconjugates is apparent because the "protein" portion of a glycoprotein will not function optimally without its carbohydrate part. Biologically important molecules that are glycoproteins include enzymes, growth factors, hormones, antibodies, and the like. Because of the diverse and important biological functions of carbohydrates, aberrations in carbohydrate synthesis, degradation, or structure may give rise to disease. Such diseases may be caused by changes in carbohydrates as a result of infections, cancer, environmental factors, chemicals or metabolic imbalance in the body's own physiological systems. Many diseases are described that are associated with changes in carbohydrate structure and abundance. These diseases include cancer and leukemia (Fukuda, M., "Cell Surface Glycoconjugates as Onco-differentiation markers in Hematopoietic Cells", Biochem. Biophys. Acta 780:119, 1985), immune deficiency (Pier, F. et al., "Altered O-Glycan Synthesis in Lymphocytes from Patients with Wiskott-Aldrich Syndrome", J. Exp. Med. 173:1501, 1991), growth and mental retardation in children (Barranger JA & RO Brady (eds) Molecular Basis of Lysosomal Storage Disorders, 1984, Academic Press, Orlando; Neufeld, E.F., "Lysosomal Storage Diseases", Ann.

Rev. Biochem. 60:257, 1991), heart disease (LaBelle, M. & R.M. Krauss, "Differences in Carbohydrate Content of Low Density Lipoproteins Associated with Low Density Lipoprotein Subclass Patterns", J. Lipid Res. 31:1577, 1990), effects of alcohol on the liver (Stibler, H., "Carbohydrate-deficient Transferrin in Serum: A new marker of Potentially Harmful Alcohol Consumption Reviewed", Clin. Chem. 37:2029,1991), arthritis (Kery, V., et al., "Urinary Glycosaminoglycan Excretion in Rheumatic Disease", Clin. Chem. 38:841, 1992), and many others diseases.

The recognition that carbohydrates are involved in normal metabolism and in disease states is a new discovery and has led to the creation of a field of science called glycobiology. Strategies for managing or treating carbohydrate-associated diseases have emphasized the use of carbohydrate pharmaceuticals (Schnaar, R.L., "Complex Carbohydrates in Drug Development", Advances in Pharmacology 23, 35, 1992).

The manner in which carbohydrates can cause or prevent disease has been established for many diseases. For example the discovery that infection-fighting white blood cells use a carbohydrate coating to target or "home-in" to damaged areas of the body (Paulson, J.C. et al., "Selectin/carbohydrate-mediated Adhesion of Leukocytes"; in: Harlan, J.M., Liu DY (eds), Adhesion: Its Role in Inflammatory Disease, New York, W.H. Freeman, 1992: 19-42; Lasky et al, Selectins: Interpreters of Cell-specific Carbohydrate Information during Inflammation, Science 258:964, (1992)) led to the description of a new kind of human disease (Etzioni A et al, Brief Report: Recurrent Severe Infections Caused by a Novel

Leukocyte Adhesion Deficiency, N. Engl. J. Med. 327:1789, (1992)).

This understanding of the role of carbohydrate mediated interactions in disease processes provides opportunities for the development of new methods and compositions for the treatment of disease. For example, human inflammatory disease may be treated by biologically "disabling" the white blood cell homing system. The disabling of these white blood cells causing inflammation may be accomplished using a naturally-occurring carbohydrate-splitting enzyme applied in a therapeutic manner to patients with overactive white blood cells. The active carbohydrate on white blood cells is a structure called "sialyl Lewis-x" which has two important sugars, fucose and sialic acid. Removal of either of these sugars or otherwise blocking their contact with other molecules will disable the inflammatory response. Such removal of sugars is normally accomplished in the body by two enzymes: one which removes the fucose called α-L-fucosidase and one which removed the sialic acid called α-sialidase. Such a strategy for disabling white blood cells using purified α-L-fucosidase and α-sialidase could be used as a treatment for patients with burns of the skin, various skin diseases, arthritis, lung disease, or any number of other medical conditions.

Thus it is of interest to provide a general technique for the treatment of a variety of diseases characterized by the existence of an undesirable carbohydrate mediated interactions by applying a carbohydrate-splitting enzyme or other therapeutic catalyst in a therapeutic manner to the patient.

Summary of the Invention

The subject invention provides methods of treating or preventing various disease conditions by interfering with a carbohydrate mediated interaction between two members of a receptor binding pair. In the subject invention, the interaction between the members of a receptor binding pair are disrupted by modifying either one or both of the binding pair members by means of a chemical reaction catalyzed by a therapeutic catalyst. A therapeutic catalyst is typically an enzyme capable of catalyzing a reaction that adds carbohydrates to, removes carbohydrates from, or modifies the structure of carbohydrates already present, on a member of a receptor binding pair that may be involved in mediating a disease condition.

The therapeutic catalyst may be administered to a subject in a variety of ways. An aspect of the subject invention is to provide for compositions for use in the subject methods, where the compositions are formulated so as to be adapted to the specific method of administration. One aspect of the subject invention is to provide for the administration of the therapeutic catalyst by parenteral administration. Another aspect of the subject invention is to provide for the administration of therapeutic catalysts by producing cellular transformation vectors containing nucleic acid sequences encoding therapeutic catalysts that are carbohydrate-modifying enzymes.

Description of the Specific Embodiments

Definitions

The term "receptor binding pair" refers to a set of two molecules having a specific, i.e., non-random, binding affinity for one another. Examples of specific binding pairs include antibodies and antigens, cell surface receptors and ligands, and the like. At least one member of a receptor binding pair may be immobilized to the surface of a cell. Additionally, both members of a receptor binding pair may be cell surface molecules, so as to provide for specific cell-cell interactions. The individual molecules forming a receptor binding pair may be proteins, nucleic acids, carbohydrates, or small organic molecules. Individual components of a receptor binding pair also may be multi-subunit proteins.

The term "carbohydrate mediated interaction" refers to a specific, i.e. non-random, physical interaction between molecules forming a receptor binding pair in which at least a portion of one of the molecules contacting the other molecule is a carbohydrate. For example, the interaction between a glycoprotein and its cognate receptor is a carbohydrate mediated interaction between receptor binding pair members. Similarly the interaction between a carbohydrate and a specific receptor for that carbohydrate is also an example of a carbohydrate mediated interaction. The interaction between receptor binding pair members may be carbohydrate-mediated interactions.

The term "therapeutic catalyst" as used herein refers to catalysts used for administration to a patient. Therapeutic catalysts may be enzymes, polynucleic acid sequences (including ribozymes), and the like. The term "therapeutic catalyst" as used

herein refers to carbohydrate modifying enzymes and other molecules capable of catalyzing a reaction catalyzed by a carbohydrate modifying enzyme. The therapeutic catalysts of the subject invention catalyze chemical reactions that may be catalyzed by carbohydrate-modifying enzymes. A substrate of a therapeutic catalyst is a member of a receptor binding pair. The therapeutic catalyst catalyzes a reaction in which a carbohydrate (including the carbohydrate portion of a glycoconjugate) is structurally modified.

The term "treating" when used with respect a disease refers to either the treatment of the disease or the prevention of the disease.

The term "carbohydrate-modifying enzyme" refers to enzymes that catalyze a reaction that may alter the structure of a carbohydrate substrate in a variety of ways including the hydrolysis of linkages between monosaccharide units, the formation of linkages between monosaccharide units, the addition of various side groups to carbohydrate molecules, and the structural alteration of a carbohydrate molecule without the addition or removal of any atoms. Classes of carbohydrate-modifying enzymes include hydrolases, lyases, acetylase, sulfatases, phosphatases, kinases, epimerases, methylases, amidases, transaminases, and oxioreductases. Carbohydrate modifying enzymes may also be identified by reference to the nomenclature system recommended by the International Union of Biochemistry; carbohydrate modifying enzymes include enzymes in classes 1.1-1.17, and various subclasses thereof, 2.1-2.8 and various subclasses thereof, 3.1-3.11 and various subclasses thereof, 4.1-4.99 and various

subclasses thereof, 5.1-5.99 and various subclasses thereof, and 6.1-6.5 and various subclasses thèreof. Examples of carbohydrate modifying enzymes include α-L-fucosidase, α-sialidase (neuraminidase), ß-galactosidase, α-galactosidase, α-mannosidase, ß-mannosidase, α-iduronidase, hexosyl sulfatase, hexosyl phosphastase, hexosylacetylase, α-xylosidase, and hexosyl epimerase.

Detailed Description of The Invention

The subject invention provides novel methods and compositions for treating a variety of diseases in humans and other mammals by administering therapeutic catalyst, i.e., enzymes or similar catalysts, capable of catalyzing chemical reactions in which the structure of a receptor binding pair member (or members) is altered by the addition, removal, or other structural alteration of carbohydrate components of a receptor binding pair member. Structurally altering receptor binding pair members that mediate a biological interaction of interest by chemical reactions catalyzed by a therapeutic catalyst the biological function of the receptor binding pair member interaction. Disruption of carbohydrate-mediated receptor binding pair interactions may have various beneficial effects, depending upon the choice of which receptor binding pair interaction is to be disrupted.

The subject invention differs substantially from many other forms of medical therapy in which an enzyme (or similar catalyst) is added to the body either directly or through genetic therapy methods because conventional therapy methods involve replacement of abnormally low levels of an enzyme

normally present in the body. The subject invention on the other hand, adds catalysts to the body so as to catalyze a chemical reaction in which the structure of a molecule is altered, whereby the biological function of the target molecule, i.e., a member of a receptor-binding pair, is destroyed, substantially reduced, or modified. Thus, the subject invention provides for the treatment of diseases not necessarily caused by a deficiency of an enzyme.

In addition to providing methods of treating or preventing disease by interfering with a mammal's own receptor binding pair interactions, the subject invention also provides for methods of treating infectious disease by interfering with receptor binding pair interactions. Many infectious diseases involve the interaction of surface carbohydrates (or glycoconjugates) present on a microorganism (or multicellular parasite) with receptors (carbohydrate or otherwise) present in a host. Additionally, many infectious diseases involve the interaction of receptors (carbohydrate or otherwise) present on a microorganism (or multicellular parasite) with carbohydrates (or glycoconjugates) present on cells in a host. These receptor binding pair interactions between a host and an infectious organism may play important roles in the inception and/or progression of various infectious diseases. Interference with the interaction of receptor binding pair members, where one of the binding pair members is present on a infectious organism, may be achieved by administering a therapeutic catalyst so as to catalyze a chemical reaction that modifies a binding pair member so as to

interfere with the interaction between binding pair members.

Some infectious organisms possess surface carbohydrates or glycoconjugates that serve to promote the infection process by providing a mechanism for evading the hosts immune system, e.g., the immunosuppressive methylated rhamnose containing glycolipid coat of Mycobacterium leprae. It is also of interest to provide therapeutic catalysts capable of catalyzing chemical reactions that modify the structure of these immune system evading carbohydrates so as to provide for their recognition by a host's immune system.

The selection of appropriate therapeutic catalysts for the treatment of a specific disease may be made based on the knowledge of the structure (or partial structure) of the carbohydrate (or carbohydrate moiety of a glyconjugate) that is a receptor binding pair member of a receptor binding pair mediating a disease of interest. Carbohydrate structures may be determined empirically or by review of the literature. A particularly useful compilation of carbohydrate structures can be found in the CarbBank Complex Carbohydrate Structure Database that may be obtained from the University of Georgia/CarbBank/CCSD 114 W. Magnolia, Suite 305, Bellingham, WA 98225.

The selection of appropriate therapeutic catalysts for the treatment of a specific disease will be apparent once the structure (or partial structure) of the receptor binding pair member of interest for modification is known. Suitable therapeutic catalysts are selected on the basis of their ability to catalyze chemical reactions capable

of structurally modifying the receptor binding pair member of interest. Numerous enzymes and other catalysts have previously been identified; by reviewing the available scientific literature, catalysts capable of using the receptor binding pair member of interest may be selected. Additionally, catalysts capable of catalyzing reactions modifying specific carbohydrate structures may be found using the methods described in U.S. patent application 07/840,739, filed February 25, 1992 and entitled "Fluorophore-Assisted Cloning Analysis" which is herein incorporated by reference.

Another aspect of the invention is to provide pharmaceutical compositions containing therapeutic catalysts. Therapeutic catalysts may be administered in a number of different ways including, topically, orally, intranasally, by injection or by inhalation in the form of a pharmaceutical compositions comprising a therapeutic catalyst in the form of the original compound or optionally in the form of a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable carrier which may be a solid, semi-solid or liquid diluent or an ingestible capsule, and such compositions comprise a further aspect of the invention. The therapeutic catalysts may also be used with carrier material. The therapeutic catalyst containing pharmaceutical compositions of the invention may be formulated so as to be adapted for different sites of administration to a subject. Examples of pharmaceutical compositions include tablets, drops such as nasal drops, compositions for topical application such as ointments, jellies, creams and suspensions, aerosols for inhalation,

nasal spray. liposomes, etc. Usually the therapeutic catalyst will comprise between 0.05 and 99%, or between 0.1 and 99% by weight of the composition, for example between 0.5 and 20% for compositions intended for injection and between 0.1 and 50% for compositions intended for oral administration.

When therapeutic catalysts are administered in the form of a subcutaneous implant, the compound is suspended or dissolved in a slowly dispersed material known to those skilled in the art, or administered in a device which slowly releases the active material through the use of a constant driving force such as an osmotic pump. In such cases administration over an extended period of time is possible.

To produce pharmaceutical compositions in this form of dosage units for oral application containing a therapeutic catalyst, the catalyst may be mixed with a solid, pulverulent carrier, for example lactose, saccharose, sorbitol, mannitol, a starch such as potato starch, corn starch, amylopectin, laminaria powder or citrus pulp powder, a cellulose derivative or gelatine and also may include lubricants such as magnesium or calcium stearate or a Carbowax® or other polyethylene glycol waxes nd compressed to form tablets or cores for dragees. If dragees are required, the cores may be coated for example with concentrated sugar solutions which may contain gum arabic, talc and/or titanium dioxide, or alternatively with a film forming agent dissolved in easily volatile organic solvents or mixtures of organic solvents. Dyestuffs can be added to these coatings, for example, to distinguish between different contents of active substance. For the composition of soft gelatine capsules consisting of

gelatine and, for example, glycerol as a plasticizer, or similar closed capsules, the active substance may be admixed with a Carbowax® or a suitable oil as e.g. sesame oil, olive oil, or arachis oil. Hard gelatine capsules may contain granulates of the active substance with solid, pulverulent carriers such as lactose, saccharose, sorbitol, mannitol, starches (for example) potato starch, corn starch or amylopectin), cellulose derivatives or gelatine, and may also include magnesium stearate or stearic acid as lubricants.

Therapeutic catalysts of the subject invention may also be administered parenterally such as by subcutaneous, intramuscular or intravenous injection or by sustained release subcutaneous implant. In subcutaneous, intramuscular and intravenous injection the therapeutic catalyst (the active ingredient) may be dissolved or dispersed in a liquid carrier vehicle. For parenteral administration the active material may be suitably admixed with an acceptable vehicle, preferably of the vegetable oil variety such as peanut oil, cottonseed oil and the like. Other parenteral vehicles such as organic compositions using solketal, glycerol, formal, and aqueous parenteral formulations may also be used.

For parenteral application by injection compositions may comprise an aqueous solution of a water soluble pharmaceutically acceptable salt of the active acids according to the invention, desirably in a concentration of 0.5 - 10%, and optionally also a stabilizing agent and/or buffer substances in aqueous solution. Dosage units of the solution may advantageously be enclosed in ampoules.

The pharmaceutical compositions are suitably in the form of an ointment, gel, suspension, cream or the like. The amount of active substance may vary, for example between 0.05-20% by weight of the active substance. Such pharmaceutical compositions for topical application may be prepared in known manner by mixing the active substance with known carrier materials such as isopropanol, glycerol, paraffin, stearyl alcohol, polyethylene glycol, etc. The pharmaceutically acceptable carrier may also include a known chemical absorption promoter. Examples of absorption promoters are, e.g., dimethylacetamide (U.S. Pat. No. 3,472,931), trichloro-ethanol or trifluoroethanol (U.S. Pat. No. 3,891,757), certain alcohols and mixtures thereof (British Pat. No. 1,001,949). A carrier material for topical application to unbroken skin is also described in the British patent specification No. 1,464,975, which discloses a carrier material consisting of a solvent comprising 40-70% (v/v) isopropanol and 0-60% (v/v) glycerol, the balance, if any, being an inert constituent of a diluent not exceeding 40% of the total volume of solvent.

The dosage at which the therapeutic catalyst containing pharmaceutical compositions are administered may vary within a wide range and will depend on various factors such as for example the severity of the infection, the age of the patient, etc., and may have to be individually adjusted. As a possible range for the amount of therapeutic catalyst which may be administered per day be mentioned from about 0.1 mg to about 2000 mg or from about 1 mg to about 2000 mg.

The pharmaceutical compositions containing the therapeutic catalysts may suitably be formulated so that they provide doses within these ranges either as single dosage units or as multiple dosage units.

In addition to containing a therapeutic catalyst (or therapeutic catalysts), the subject formulations may contain one or more substrates or cofactors for the reaction catalyzed by the therapeutic catalyst in the compositions. Therapeutic catalyst containing compositions may also contain more than one therapeutic catalyst.

The therapeutic catalysts employed in the subject methods and compositions may also be administered by means of transforming patient cells with polynucleic acids encoding the therapeutic catalyst when the therapeutic catalyst is a protein or ribonucleic acid sequence. The therapeutic catalyst encoding sequence may be incorporated into a vector for transformation into cells of the subject to be treated. The vector may be designed so as to integrate into the chromosomes of the subject, e.g., retroviral vectors, or to replicate autonomously in the host cells. Vectors containing therapeutic catalyst encoding nucleotide sequences may be designed so as to provide for continuous or regulated expression of the therapeutic catalysts. Additionally, the genetic vector encoding the therapeutic catalysts may be designed so as to stably integrate into the cell genome or to only be present transiently. The general methodology of conventional genetic therapy may be applied to polynucleotide sequences encoding therapeutic catalysts. Reviews of conventional genetic therapy techniques can be found, among other places in, Friedman, T. Science

244:1275-1281 (1989), Ledley, F. D. J. Inher. Metab. Dis. 13:587-616 (1990), and Tostoshev and Anderson, Curr. Opinions. Biotech. 1:55-61 (1990)

The invention having been described in the preceding paragraphs is illustrated by the following example. The following example is offered for the purpose of illustrating, not limiting, the subject invention.

EXAMPLES

Example 1

ISOLATION OF THERAPEUTIC CATALYSTS

Cloning of Human α-L-fucosidase

The Human α-fucosidase gene was cloned form Human liver poly-A RNA by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) techniques. Two sets of PCR primers were designed by inspection of the published sequence (Fukushima H et al, Proc. Natl. Acad. Sci. USA, 82:1262-1265, 1985) of Human liver α-fucosidase. The first set (5' set) consisted of the following sequences: 5' Forward (5' GCTCTAGAATTATGGTGCGTCGGGCC-CAGCCTCCGCGC-3') 5' Reverse (AATGCTGGTGCACATCTCCCA 3'). The second set (3' set) consisted of (5' TGGGAGATGTGCACCAGCATT 3') and 3' Reverse (5' CGGGATCCTTACTTCACTCCTGTCAGCTTTAT 3').

Human liver polyA RNA was converted to cDNA by priming with random hexamers and treating with reverse transcriptase as recommended (Sambrook et al, in, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, 1982) for the 5' part of the gene. For the 3' part of the gene, the RNA was primed with the 3' Reverse primer and treat as above. Following cDNA synthesis, the 5' and

3' sets of primers were added to their respective cDNA reactions and PCR was performed with the following protocol:

1. 95 C 5min.
2. 60 C 1min., 72 C 3 min., 95 C 30s. 30 cycles
3. 72 C 7min.

Following amplification, the DNA from the 5' set of primers (5' DNA) was cleaved with restriction enzymes XbaI and ApaLI. The DNA amplified with the 3' set of primers (3' DNA) was cleaved with BamHI and ApaLI. Both sets of DNA fragments were then ligated into plasmid pT7-3 (Miller H et al Bio/Technology 7:698-704, 1989) which has been cleaved with Xba and BamHI. The clones were electroporated into strain BL21 (DE3) (Studier WB and BA Moffat J. Mol. Biol. 189:113, 1986) and screened for production of active fucosidase using PNP-fucose as a substrate. Clones were identified that produced active Human α-fucosidase.

Cloning of α-sialidase

Sialidase (Neuraminidase) from Clostridium perfringens was cloned by PCR directly from Clostridium DNA. By inspection of the DNA sequence of Clostridium sialidase gene (Roggentin P et al, FEBS Letters 238:31-34, 1988), the following primers were designed: Forward (5' GCTCTAGAATTATGTTTGAAAAGAACCTAGATATAAGCC 3'), Reverse (5' CGGGATCCTTATTGTTTATTAATAGTGAG 3').

The sialidase gene was amplified by the following protocol:

1. 94 C 1min.
2. 94 C 30sec., 55 C 30sec., 65 C 1min. 5 cycles.
3. 94 C 30sec 65 C 1 min, 25 cycles.
4. 65 C 5 min.

After amplification, the DNA was cleaved with Xbal and BamHl and ligated into plasmid pT7-3 and electroporated into strain BL21 (DE3). The colonies were screened for active sialidase activity using methylumbelliferyl-sialic acid. Positive clones were chosen.

The isolated human neuraminidase and fucosidase genes are used to produce neuraminidase and fucosidase, respectively, from genetically engineered host cells. Neuraminidase and fucosidase were used to destroy, i.e., structurally modify, sialyl-Lewis x molecule. Fucosidase and neuraminidase, separately, and in combination with one another, were incubated with sialyl-Lewis x. The structural modification of the sialyl-Lewis by the enzymes was monitored by fluorophore-assisted carbohydrate electrophoresis. Destruction of sialyl-Lewis x was observed in the reaction mixtures containing either one or both of the tested enzymes. As specific binding pairs comprising sialyl-Lewis x are involved in the inflammation response, the experimental results indicate that fucosidase and neuraminidase can be used as therapeutic catalysts for the treatment of inflammation.

Example 2

FERTILIZATION/CONTRACEPTION

A galactose at the non-reducing terminus of O-linked oligosaccharides in mouse egg zona pellucida glycoprotein ZP3 is essential for the sperm receptor activity of the ZP3 glycoprotein, see Wassarman, P.M., Proc. Natl. Acad. Sci. USA 85: 6778-6782 (1988). An enzyme capable of hydrolyzing the galactose at the non-reducing end of the ZP3 glycoprotein is administered to mammals in the form of a slow-release intra-vaginal suppository. The reaction catalyzed by the enzyme prevents the productive interaction between sperm and egg, thereby reducing fertility.

Example 3

CANCER THERAPY

Human tumor cells are known to produce a variety of gangliosides, e.g. G_{M2} and G_{M3}, that are present in high concentrations and have an immunosuppressive effect on the cell surface, for example see Ladisch et al., Biochimica et Biophysica Acta 1125: 180-188 (1992), Grayson and Ladisch, Cellular Immunology 139: 18-29 (1992). A neuroblastoma is treated by injecting G_{M2} and G_{M3} sialidases into a patient. Neuroblastoma patients are also treated by placement of a controlled release implant containing G_{M3} and G_{M2} specific sialidases at the site of the tumor. The reaction catalyzed by the sialidase(s) alter the structure of the gangliosides so as to provide for enhanced recognition of the tumor cells by the immune system.

Example 4

REDUCTION OF SCARRING

Heparin has been shown to potentiate the growth stimulatory effects of acidic fibroblast growth factor, see Gospodarawicz, P. and Cheng J., J. Cell. Physiol. 128: 475-484. The degree of sulfation of heparin and the length of the heparin chains has been shown to be important for the productive interaction between heparin and acidic fibroblast growth factor, see Sudhalter et al., J. Bio. Chem.264: 6892-6897 (1989). The growth stimulatory effects of fibroblast growth factor can lead to the generation of scar tissue from both accidents and surgery, as well as other forms of trauma to the skin.

The formation of scar tissue is reduced by topically applying an ointment containing a sulfatase to the site of plastic surgery. The sulfatase containing ointment is applied repeatedly so as to ensure that a high percentage of non-denatured enzyme is present at any given time.

Example 5

IMMUNE SYSTEM ENHANCEMENT

Many infectious organisms are able to escape detection by a host organisms immune system by shielding themselves with immunosuppressive (or immune system unrecognizable) carbohydrates or glycoconjugates. The bacterium Mycobacterium leprae, the etiological agent of leprosy, is covered with an immunosuppressive phenolic glycolipid having an oligosaccharide structures of 3-O-Me-rhamnose,2,3-di-O-Me-rhamnose, and 3,6-di-O-Me-glucose, see Hunter et al., J. bio. Chem. 257: 15072-15078 (1982), and Kaplan et al., Proc. Natl. Acad. USA 86: 6269-6273 (1987). Mycobacterium tuberculae, the etiological

agent of tuberculosis possesses the same immunosuppressive glycolipid. Removal of the O-methyl from the rhamnose constituent of the phenolic glycolipid has been shown to abolish the immunosuppressive effect. (Brennan) [Same Ref. as Hunter??]

A patient suffering form leprosy or tuberculosis is intravenously administered a solution containing the enzyme rhamnose methylase, which clears the methyl-rhamnose moiety from the immunosuppressive glycolipid on the infectious organism. Side effects are minimal because of the absence of methyl-rhamnose in human cells. The treatment is supplemented by administration of antibiotics known to be effective for the treatment of tuberculosis or leprosy, accordingly.

Example 6

COAGULATION ENHANCERS

Excessive bleeding is a common and serious problem in victims of trauma, cancer patients, patients undergoing anti-coagulant therapy, and others. It has been shown that the anti-coagulant properties of the naturally produced anti-coagulant heparin is dependent of the thrombin binding regain of heparin, a pentasaccharide having the structure -GlcNAc(6-OSO_3)-GlcA-$GlcNSO_3$(3,6-di-OSO_3)-IdoA(2-OSO_3)-$GlcNSO_3$(6-OSO_3)-, see Oscarsson, et al., J. Biol. Chem. 264: 296-304 (1989). The important structural feature of the pentasaccharide structure required for anticoagulant activity have been analyzed by the synthesis of biologically active structural analogs, see Wessel et al., Helvetica Chimica Acta 72: 1268-1277 (1989); Boeckel et al., Angew. Chem. Int. Ed.

Engl. 27: 1177. Thus the process of coagulation may be disrupted by altering the structure of heparin in vivo.

A patient suffering from internal bleeding caused by an excessive level of heparin (or attributable to other causes) is treated by intravenous administration of an iduronrate sulfatase solution. The sulfatase acts as a therapeutic catalyst, catalyzing a reaction that structurally modifies heparin so as to lower its biological activity, thereby promoting clotting and reducing internal bleeding.

Equivalents

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. Indeed, various modifications of the above-described modes for carrying out the invention which are obvious to those skilled in the field of carbohydrate biochemistry, enzymology, or related fields, are intended to be within the scope of the following claims.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Glyko, Inc.
81 Digital Drive
Novato, California 94949
United States of America

(ii) TITLE OF INVENTION: Methods And Compositions For Treating Diseases With Carbohydrate Modifying Enzymes

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Pennie & Edmonds
(B) STREET: 2730 Sand Hill Road
(C) CITY: Menlo Park
(D) STATE: California
(E) COUNTRY: U.S.A.
(F) ZIP: 94025

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US PCT/US94/04464
(B) FILING DATE: 22-APR-1994
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Halluin, Albert P.
(B) REGISTRATION NUMBER: 25,227
(C) REFERENCE/DOCKET NUMBER: 8133-023

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (415) 854-3660
(B) TELEFAX: (415) 854-3694
(C) TELEX: 66141 PENNIE

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCTCTAGAAT TATGGTGCGT CGGGCCCAGC CTCCGCGC 38

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AATGCTGGTG CACATCTCCC A 21

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGGGAGATGT GCACCAGCAT T 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGGGATCCTT ACTTCACTCC TGTCAGCTTT AT 32

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCTCTAGAAT TATGTTTGAA AAGAACCTAG ATATAAGCC 39

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGGGATCCTT ATTGTTTATT AATAGTGAG 29

CLAIMS

1. A method of treating a disease or disease condition involving the interaction of a receptor binding pair composed of a first binding pair member and a second binding pair member having a binding affinity for said first binding pair member, said method comprising the step of,

adding an effective amount of a therapeutic catalyst capable of catalyzing a reaction in which the structure of the at least one of said binding pair members is modified so as to reduce the binding affinity between said first binding pair member and said second binding pair member.

2. A method according to Claim 1, wherein said catalyst is an enzyme.

3. A method according to Claim 1, wherein said catalyst is a polynucleic acid.

4. A method according to claim 2, wherein said first binding pair member is a carbohydrate.

5. A method according to Claim 4, wherein said carbohydrate contains at least one saccharide linkage and wherein said enzyme is capable of catalyzing the hydrolysis of at least one of said saccharide linkages.

6. A method according to claim 2, wherein said first binding pair member is a glycoconjugate.

7. A method according to claim 6 wherein said glycoconjugate is a glycoprotein consisting of a protein portion and a carbohydrate portion.

8. A method according to Claim 6, wherein said carbohydrate portion contains at least one saccharide linkage and wherein said enzyme is capable of catalyzing the hydrolysis of at least one of said saccharide linkages.

9. A method according to Claim 4, wherein said enzyme is capable of catalyzing the addition of at least one least one saccharide subunit to said carbohydrate.

10. A method according to Claim 6, wherein said enzyme is capable of catalyzing the addition of at least one saccharide subunit to said carbohydrate portion of said glycoprotein.

11. A method according to claim 1, wherein said disease is an inflammatory disease.

12. A method according to claim 11, wherein said adding step comprises the steps

transforming patient cells with a nucleic acid sequence encoding said enzyme, whereby transformed patient cells are produced.

13. A method according to claim 11, wherein said nucleic acid sequence is present on a viral vector.

14. A method according to Claim 11, wherein said viral vector is applied topically.

15. A method according to claim 1, wherein said disease is selected from the group consisting of burns, skin diseases, eye disease, genitourinary diseases, alimentary diseases, central nervous system diseases, peripheral nervous system diseases, blood diseases, immune diseases, and infectious diseases.

16. A method according to Claim 2, wherein said enzyme is selected from the group consisting of α-L-fucosidase, α-sialidase (neuraminidase), ß-galactosidase, α-galactosidase, α-mannosidase, ß-mannosidase, α-iduronidase, hexosyl sulfatase, hexosyl phosphastase, hexosylacetylase, α-xylosidase, and hexosyl epimerase.

17. A composition suitable for administration as a drug comprising a carbohydrate modifying enzyme.

18. A composition according to claim 16, wherein said composition is formulated for topical or parenteral administration.

19. A composition according to claim 17, wherein said composition is for the treatment of a disease selected from the group consisting of burns, skin diseases, eye disease, genitourinary diseases, alimentary diseases, central nervous system diseases, peripheral nervous system diseases, blood diseases, immune diseases, and infectious diseases.

20. A composition according to Claim 17, wherein said enzyme is selected from the group consisting of α-L-fucosidase, α-sialidase (neuraminidase), ß-galactosidase, α-galactosidase, α-mannosidase, ß-mannosidase, α-iduronidase, hexosyl sulfatase, hexosyl phosphastase, hexosylacetylase, α-xylosidase, and hexosyl epimerase.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 94/04464

A. CLASSIFICATION OF SUBJECT MATTER

A 61 K 37/48

According to International Patent Classification (IPC) or to both national classification and IPC 5

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A 61 K 37/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 117, no. 1, issued 1992, July 06 (Columbus, Ohio, USA), D.M. GOLDBERG "Enzymes as agents for the treatment of disease", page 5, column 1, no. 96v; & Clin. Chim. Acta 1992, 206(1-2), 45-76 (Eng). --	1-20
P,A	WO, A1, 94/01 137 (HYBRITECH INC.) 20 January 1994 (20.01.94), claim 10. --	1,16
A	CHEMICAL ABSTRACTS, vol. 101, no.17, issued 1984, October 22 (Columbus, Ohio, USA), P.I. KABACHNYI "Current	1

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 August 1994	07. 10. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	SCHARF e.h.

Form PCT ISA 210 (second sheet) (July 1992)

C.(Conunuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	aspects of the use of amylolytic enzymes for the diagnosis and treatment of gastrointestinal disease and the present status of their production", page 4, column 1, no. 143 354h; & Farm. Zh.(Kiev) 1984, (4), 25-32 (Ukrain). ----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 94/04464

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-16
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-16 are directed to methods of treatment of the human body, the search has been carried out and based on the alleged effects of the compounds.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.